

inhibitor.

N-379

5/8/46.

Blend 368-7 (43-3 mycelium, no exc. leuc.) ~~mixed~~, steadily, 11PQ
Transfer remainder of blendate to sterile flask + store cold.

1. 5 ml unblended medium - 43-3

2. " " - 10A

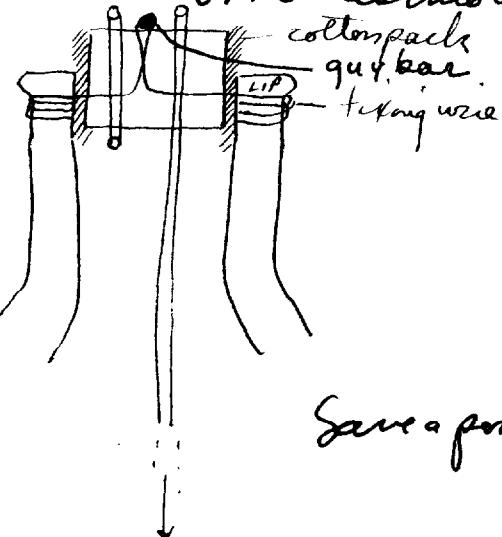
Add 5 ml extract + 5 ml F(0) + ascorb. 1 mg dl-leucine

	nox.	P11.	P13	P22
3 ext. + F(0)	-	-	±	±
4 "	10A	+	+++	++++
5 "	43-3	-	±	±
6. "	" + leuc	-	++	++
7 "	" " 10A	++	++	++++
8.	" " 43-3.	++	++	++

No evidence of inhibition.

Production of Neurospora. 5/14/46.

by 20 l. Pyrex racking, 10l. of a Fries \bar{c} 2% glucose,
5v/l desthiobiotin. $D_{bro} \bar{c} 8.47$



Inoculate 6/8/46. 12 M.
Harvest A12

Yield: ca 150-200g. dry

Send to Graf in 95% alcohol (before drying)

Save a portion and dry for hydrolysis.

Mutants by selection.

N-381

5/14/46. 349 - A5 x 25a.

5/14-20 isol. Color Morph. F(0). Isolate ascospores, 1/ perithium. 7 gen. 120.

1	n.g.		
2	"		
3	"		
4	"		
5	"		
6	+	+	++
7	n.g.		
8	n.g.		
9	n.g.		
10	n.g.		
11	n.g.		
12	-	+	++
13	+	+	++
14	+	-	
15	n.g.		
16	-	+	++
17	+	+	++
18	n.g.		
19	+	+	++
20	n.g.		

Iso genetic N - stocks
Isolations.

N 382

5/28/46 SY7 x 360-6. (S series.) 4/30 photograph

A. 1^{nq}
2"
3
4
a/ 5^v
6^{nq}
7
8"
9
10."
11.

G(0)

SY7 x 1633a (70-26) 4/30 p pale

21	-	
22	+	
23	+	
✓ 24	-	
25	-	
26	+	
27	n.q	
28	nq	+
29	n.q.	+
30	n.q.	

37401a x SY 1

378-3 x SY7

SY7 x 378-3 5/10 isolated

a	31	-	
a	32	-	store
A ✓	33	+	
	34	-	
	35	+	
	36	+	
	37	+	
	38	nq	
	39	nq	
	40.	nq	

382-52

N-582
a

nic a + put A
4540 5531.

dated 4/28. Isolate:

5/14: ~ o nic put weight
infestile.

1
2
3
4
5
6
7
8
9
10
11

~~more germinated.~~

Reheat.

	Color	F(0)	366 - 3 x 25g. (Lc-4637+ Lc -15300). Only 6 gms!
#41 is giant pore	+	++	
n.g.	- (pf)	"	
21	+	"	
22	+	"	
23	+	"	
24	+	"	
25	+	"	
26	± (dil)	"	

water
go far!

multiple mutants

N-383

4540a \times 370-12 (4540-5531 A). dil)

5/18/46 - G (nic) G (pnt) G (post-nic) color Sex.

fluffy	1	n.g.	-	-	+	+	\pm	A	most spores are not colored.
	2	-	-	-	+	+	+	a	Sp. A. select for deep pigment.
	3	-	-	-	+	+	+	a	Sp. B. " " light " .
	4	-	-	-	-	-	-		
A ✓	5	-	-	-	+	+	-	A	
	6	-	-	-	+	+	+	a	
	7	"							
	8	"							
	9	"							
	10	"							
	11	"							
	12	"							
	13	"							
	14	"							

21
22
23
24
25
26 N
27
28
29

5/18/46. pnt

~~lc~~ - post-lc. Sex.
5531 A \times 33757 a.

31
32
Random 33
34 ✓
35
36
37

border
41° + -
42° + -
43° + -
44° + +
45° + +
46° + +
47° + +
48° + +

??

51°
52°
53° + -
54°
55° + +
56° + +
57° - +

border

1633a x SY7A.
58-6 x SY7.

N-383a

4/30-5/14.

5/14. -
all spores
colorless.

~~366-3 x 33757a.~~

G(0). 58-6 x SY7.

Used more isolates.

5/14:
several per. 3 sporangia
sporangia P1. 1 N.G.
2
3

12 P1
4 P2
5
6

P3 7 N.G.
8 +
9 +

P4 11 +
12
13

P5 14 +
15
16

P6. 17
18
19

20 +

PAB - adaptations

384

358-6 x 347A

5/28/46

6(0).

Random -	1	+	
from	2	+	present
spore	3	+	
punct.	4	+	
	5	+	
29 card.	6	+	
	7	+	
	8	+	
184.9. 1st	9	+	
heating	10	+	
	11	+	
all morph.	12	+	
9K. compare			
microscopic			
retention			

Isolate penicillium as the possible of other spores. However some had discharged. 5/18/46 Test on F(0) A31.
Color. F(0).

371-11. 1	+	+
P:	2	+
SS31-15300+	3	+
37401	4	-
X	5	+
SS31-37401	6	+
-	7	+
	8	+
	9	+
	10	-
	11	+
	12	+
	13	+ +
	14	+
	15	+ -
	16	+
	17	+ 64
	18	+
	19	+
	20	+
	21	+
	22	+ ^{ng}
	23	+ ^{ng} / ³⁺ ₁₈₋
371-12	31	+
do.	32	+
	33	-
	34	+
	35	+
	36	\pm
	37	\pm
	38	+
	39	+
	40	+
	41	+
	42	+

	Color	F(0)	F(pit)
371-1551	+	+	+
52	+	+	+
P: 5331- 53	\pm	+	+
37401 54	+	+	+
X 55	\pm	+	+
SS31-15300 56	+	+	+
+ 57	+	- +	+
37401 58	\pm	+	+
interoc. 59	+	+	+
60	+	+	+
61	+	+	+
62	+	+	→ slow ++. ??
371-16 71	+	-	+
5331-37401 72	+	-	+
X 73	+	-	+
SS31-15300 75	+	-	+
and 76	+	-	+
37401 77	+	-	+
unsp. 78	+	-	+
conid. 79	+	-	+
at first 80	+	-	+
81	+	-	+
82	-	-	+
83	+	-	+

Why should ~~not~~ conid. be more effective?

Nuclear origin, etc.

N 386.

6/1/46

* Proc. F(0) plates in 4545a + 37401a. for heterocaryon formation.

P1. No growth. (dead culture?)

P4. Repeat in fresh cultures.

P6 - no heterocaryotic growth!!! see 389. compare

L_1 and L_2 ; $L_1 \bar{\nu} + L_2^+$. known
situation.

6/1/46. 25° drop 4P.

Use 43-3 as L_2^- 368-22 as L_2^+ . 10A as L_1^+ $L_1^- = 10 - 4637A$.
5P1.

Drop N ∞ plates & the following in pairs, in end plate ring Dg.
all OK.

1. $L_2 \bar{\nu} + L_1^+$

pres. note.

2. $L_2 \bar{\nu} + L_1^-$

3. $L_2^+ + L_1^+$

4. $L_2^+ + L_1^-$

5. $L_2^{\checkmark +} + L_2^{\checkmark -}$

3PM 6/2/46

Isolate 387 hyphal tips toward medium. α is solid; \circ is liquid.Incubate at 30°. α 1. 1 to ∞ color G(0)-color.

L_1 +	L_2 -	1 to ∞ color	G(0)-color.
81		+	
82		+	
83		+	
84		+	
85		+	
86		+	

L_1 +	L_2 -	1 to ∞	
11	++	-	
12	"	-	
13	"	-	
14	"	-	
15	+	-	
16	++	-	

L_1 -	L_2 -	1 to ∞	
21		-	
22		-	
23		-	
24		-	
25		-	
26		-	

L_1 +	L_2 -	1 to ∞	
31		-	
32		-	
33		-	
34		-	

No.	posn.	1 to ∞	
41		+	
42		+	
43		+	
44		+	

L_1 -	L_2 +	1 to ∞	
51	2	-	
52		-	
53		-	
54		-	
55		-	
56		-	

L_1 -	L_2 +	1 to ∞	
61		++	
62		"	
63		"	
64		"	
65		"	
66		"	

5 to ∞ Color. G(0)-Color.

71	\pm	+
72	\pm	+
73	\pm	+
74	\pm	+
75	\pm	+
76	\pm	+

L_1	5 to ∞	
81	++	-
82	-	-
83	++	-
84	"	+
85	"	++
86	"	++

77

+

The color line
is quite deep.
Unless a further
mutation of 15300
is considered, this
stock is highly
suspect. Again,
should be rejected.

See:

6/4/41. Broc F(0) plate \bar{x} 94.4 + 37401 a. See edges of plate
for controls.

P6 - no growth!

Repeat P10.

- initial hyphae, but no extended growth!

In vitro activity: synthesis of pantothenic acid by *Mucor sporae*.

N 391

6/5/46.

50 ml Fries +

bac 5471030415.

1. -

2. 1 mg β -alanine + 1 mg pantothenone.

Harvest: 11 P.M. (6 $\frac{1}{2}$ days).

A. Medium

B. Mycelium in 10 cc H₂O. Then ~~still~~ boil, and remove mycelium for digestion.

Use medium 50% in assay; extract B. 10 ml/50 Fries.

bac 5531. 12 N 12.

1. F(0) 50 ml.

2. 1A.

3 1B

4 2A

5 2B.

moderate
response in
2 days

33751 - Selections v.s. domunciae.

N-392

9 JUN 1950

In previous experiments, color markers were used; here another biochemical mutant gene is employed: 33757-4540 + other single mutants.

361-6 (in F(0) plates + 1mg dl leuc. / 10 ml: P10:
is 33757-4540A).

~~A~~ "P10. Isolate 4P11.

X 361-6 + 5531A.

B 361-6 + 16117A.

Y. 361-6 + 1633-15300A.

To F(lc) small lig. hyphae

1230A13 1130A13

V	1	-
	2	-
	3	-
	4	-
	5	-
	6	-
	"	-
X	12	-
B	13	-
	14	-
	15	++
	16	-
	21	-
	22	-
	23	-
	24	-
	25	-
	26	-

To F(0).

1230A13

51	-
52	-
53	-
54	-
55	-
56	-
61	-
62	-
63	-
64	-
65	-
66	-
71	-
72	-
73	-
74	-
75	-
76	-

To Noo slants.

15, 62, 66 to N=0 large slants.

A 24. test on: F(0) F(lc) F(lcnic) F(flc)

15
62
66

B	91	+
	92	+
	93	+
	41	+++
	42	+++
	43	+++

Selection vs. Dominance

N-393.

Test 392 on le, nc, etc.

There may have been deficient
leucine in the α medium.

	le	nc	lc-nc
81	+	(±?)	+
82	+	(±?)	+
83	-	+	+
91	+	+	+
92	+	+	+
93	+	+	+
41		+	+
42		+	+

check.

N-394.

holosporic hetero angles.

6/13/46. 13 JUN 1946

12 N.

See N-83. N. strophula 299A + N. cæsarea 1633-15300A (70-27). on F/0 plate

Isolate hyphal tips to minimal liquid. 4P14.

1	—
2	—
3	—
4	—
5	—

Take a block of agar-cyclohexim & inoculate F/0/plate:

II. a few hyphae grew out and covered plate. Enidig spottily white & colored in various areas.

Syntrophyism? or unstable hetero angles?

Heterocaryon transformation
nic expt.

N-395

1 JUN 1970

1. More $F(0)$ \approx 5531A + 4540A. 1130P14.

3P15. Isolate hyphae to $F(0)$ ~~in~~ 1 ml tubes.

1
2
3
4
5.

1A16. More prot-cornmeal \approx 383-3

Chlamydomonas neopertii

A-1

A transpondon Prospect St. was found to bloom after every rain.

Collected 5/18/46, and found Stichococcus, Bacillariae and a variety of Chlamydomonads. Purify by centrifuging + photolysis, and more.

into Moris's.



P22 - some green growth noted - a few filaments + some sediment

A24 - micro exam. showed numerous flagellates. Plan to streak out when culture is heavier.

P1 - streak out on Moris's agar. + moi. liq. in culture

Eudomyces - Killing & Virents

- 36. hour culture in F(0). Shaken at 30°.

radiate in quartz tube 2 min., etc. Kroc. coli CM plates

1. Control. dil. 1:25⁽¹⁾,
 $\text{dil. } 1:10 \text{ dens. } = 5 = 89 \text{ } d = 0.51$ $1:125,000 - 29 - 36,000,000.$
 $d_{\text{orig}} = 505 \text{ } G_{\text{on.}} 31$

2. irradiated 2 min. 1 ml

$1:50$
 $1:2500$
 $1:125000$
 $1:625000$ (II) 275,000 $S = .0076$
 $p_{\text{Survival}} = 2.1$

✓ 3. 1 ml to colic N. 6 P 26.
 colic CM plates. $d = 1340 \div 95 \times 10^6 = 2 P 28.$ Dilute to 10^{-6} appear.

Complete {
 1. Spread 1cc over surface.
 2. Embed in agar.
 3. Surface agar. T(50)
 4. Spread 1cc over surface.
 5. Escolip procedure. (Navy)

Fairly uniform. Layer on surface w.g. spreading growth. and appears plate.

F(0). {
 6. Spread 1cc over surface " colonies. Pl. Layer is complete 1P.2.
 7. Embed in surface agar. 2A30.
 8. Surface agar. " not up yet. Nothing carries up
 9. Embed & covers heavily. to 38°. 7 colonies!

$pS = -\log \text{survival}$ This should be a dose.
 $= \log (1/1 - \text{killing})$

Schizosaccharomyces octosporus (S0). 611

1. Vitamin Requirements.

1. Th(O) + Biotin, Thiamin, Riboflavin, niacin, pnt, mositol.

1.

2. - biotin

3. - thiamin

4. - riboflavin

5. - nic P.J.

6. - pnt

7. - mos.

8. - pab

9. - folic

10. - B6

6/5. 1 colis 6/6
+++

unknown factors?

F) 2 HC+V ±

Any more, octosporus is genetically not
satisfactory for mutant production as it di-
ploidizes very readily. Use *S. pombe*
which requires biotin, nic, pnt + mositol.
Compare *S. cerevisiae* which does not req. pnt

3 HC ±

4. V ±

S. pombe. - misc. prelim. data.

114

1/7/46.

1 plating density. susp. in H₂O. G = 93. ≈ 31.5 d.u.

Hemacytometer count.. 137, 138 = 137 / 10⁻⁴ ml.

∴ 1 density unit = 4.3 × 10⁴ / ml. = 1.4 × 10⁶ / ml.

Vitamin Requirements - stated as biotin, pnt, nic, mox.

		Pq.
1. Fries + vits.	++	+++
2. " + 4 vits.	+	++
3 - pnt	-	++
4 - nic	-	-
5 - mox.	-	-
6. T + vits.	+	++

123047. Broz col. S. 37° sh. - growth only fair worse than
use 10 vits. outfit 1st. F

1 A9 broz.
1 - B₁ (~~Biotin Free~~)
2 - B₂

3

4

5

6

7

8

9

10

See 45.

50 ml 11. F(vits.)

37° shaking.
37° s shaking.

Vitamin Requirements
S. panche.

A-5

9 JUN 1946

9 JUN A-1

Campace A-1.

37°.

Fries + vitamin supplement: 10B-vits. - 1nd. F conts. leothi brot.

11 P 9 10 A 10

	(+)	(++)	(put)	put
1 - B ₁	+	++	nic	nic
2 - B ₂	+	++	nic	nic
3 - pab	+	++	nic	nic
4 - nic	-	-	nic	nic
5 - nic	+	++	nic	nic
6 - B ₆	-	++		
7 - nic	-	+		
8 - chol	+	++		
9 - inos.	-	-		
10. - Biotin	+	++		

Campace shaking 5 unsterile cultures. 37°.

11. F-vits	sh.	+	Growth is poorer than in tubes.
12. "	unsh.	-	
13 colic	sh.	+	Relatively anaerobic conditions
14. "	unsh.	-	

Vitamin req.

A 10.

- Fries:
 21 put inos, nic, folic (biotin)
 22 - put
 23 - folic
 24 - folic

For colony type, plate A 4-2 into F(P) = Fries + panche vits

$$6 \text{ 1:10} = 78^2 \quad d = 1051 \quad (45,000,000) \quad \text{dil. } \underline{10^6}$$

P 13 colonies finally noted. Resemble bacterial colonies.

Morulium?? too old.

PAB - responses.

E2

8/20/46.

S. + H. - 100% P.

S. + H. - 100% P.

I PAB
span = 0% / Transmission 36 hrs

	other	0	.001	.003	.01	.03	.1	.3	1
11	100	100	100	100	100	100	100	100	100
Y44	H	100	24.1	18.2	17.2	16.1	16.1	16.1	16.1
L 373-374	O	100	100	12.1	12.1	12.1	12.1	12.1	12.1
L 375-376	O	100	100	16.2	16.2	16.2	16.2	16.2	16.2
L 376-462	O	100	100	17	15	15	15	15	15

IV Glutam. acid Y44 (#17)

	0	10	20	30	40	50	60	70	80
blank	0	10	20	30	40	50	60	70	80
Y44	0	10	20	30	40	50	60	70	80

V S. + H. #22

IV Glutam. acid #11 Y30

	0	.001	.003	.01	.03	.1	.3	1
75	0	91.3	92.1	92.1	93	90	90	90
100	100	82.5	88.1	93.2	91	93	93	93
Levogl.	# 9							

(82)